TITLE: Role of sucrose-derived exopolysaccharides in *Streptococcus sanguinis* evasion to complement-mediated immunity

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ABSTRACT: *Streptococcus sanguinis* is a commensal bacterial species capable to initiate tooth colonization and to inhibit the growth of pathogenic species in dental biofilms. *S. sanguinis* is further associated with opportunist cardiovascular infections, likely due to its persistence in the bloodstream by mechanisms as yet unknown. *S. sanguinis* strains secretes glycosyltransferase P (GtfP) to synthesize exopolysaccharides (EPS) from sucrose. The aim of this study was to evaluate the role of sucrose-derived EPS by S. sanguinis strains in evasion to complement-mediated opsonophagocytosis by peripheral neutrophils (PMN), a major immune arm of blood clearance. To that purpose, we compared deposition of C3b complement protein between a GtfP-defective mutant (SKgtfP) with parent strain SK36. We also compared C3b deposition between strains isolated from the oral cavity (SK330 and SK353) and from the bloodstream (SK678 and SK1056). The strains were grown in a chemically defined medium (CDM, 37 ^{o}C, 10% CO_2), supplemented or not with 1% sucrose, until the mid log-phase of growth (A_{550nm} 0.3). Afterwards, bacteria were harvested, washed twice with PBS and incubated with normal human 20% (v/v) serum in PBS (37^{o}C, 30 min.) for C3b deposition. Cells were then washed twice with PBS and C3b labeled with FITC-conjugated anti-human C3 antibody (4^{o}C, 40 min.). Amounts of surface-bound C3b were determined by flow cytometry and expressed as geometric mean of fluorescence intensity (MFI). Opsonophagocitosis by PMN isolated from peripheral blood was determined after PMN exposure (37^{o}C, 5 min.) to FITC-labeled bacteria (MOI 200:1) and frequencies of PMN with internalized bacteria assessed by flow cytometry. SK36 strain grown in sucrose-supplemented CMD showed 2.3-fold reduction in C3b deposition and 2-fold reduction in opsonophagocytosis when compared to cells grown in CDM without sucrose ($p<0.05$). Significant reductions in C3b binding (1.7 to 2.2-fold changes) and opsonophagocytosis (2.2 to 5.3-fold changes) were also observed in oral and blood isolates grown in sucrose-CDM compared to sucrose-free CDM cultures. Additionally, deletion of *gtfP* abolished the effect of sucrose in C3b deposition or opsonophagocytosis. These findings reveal that *S. sanguinis* evasion to complement-mediated opsonophagocytosis relies on production of EPS from sucrose by GtfP.

Keywords: *Streptococcus sanguinis*, exopolysaccharide, GtfP, complement system, neutrophil.

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